



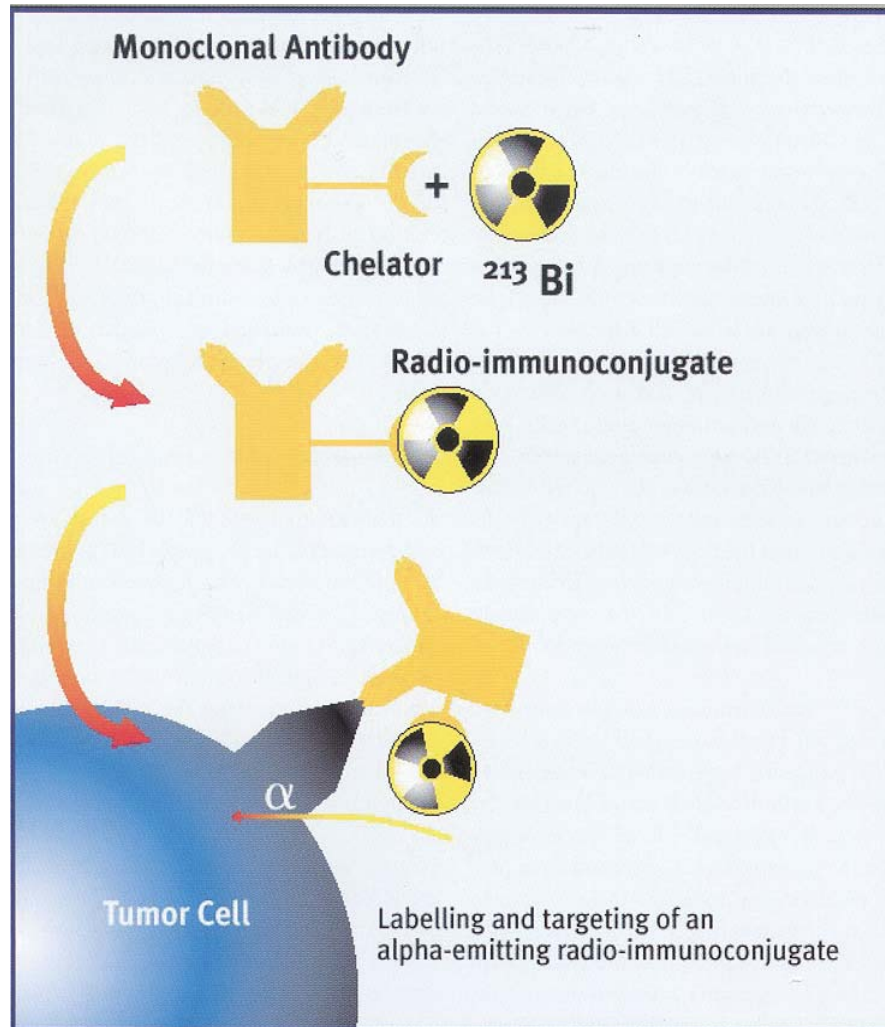
# Alpha-Immunotherapy

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European Commission, Joint Research Centre,  
Institute for Transuranium Elements,  
Karlsruhe, Germany



## Principle of Alpha-Immunotherapy



Specific recognition of cancer cells  
**Vector/Carrier**  
(e.g. monoclonal Ab's, Ab-fragments, peptides)

+

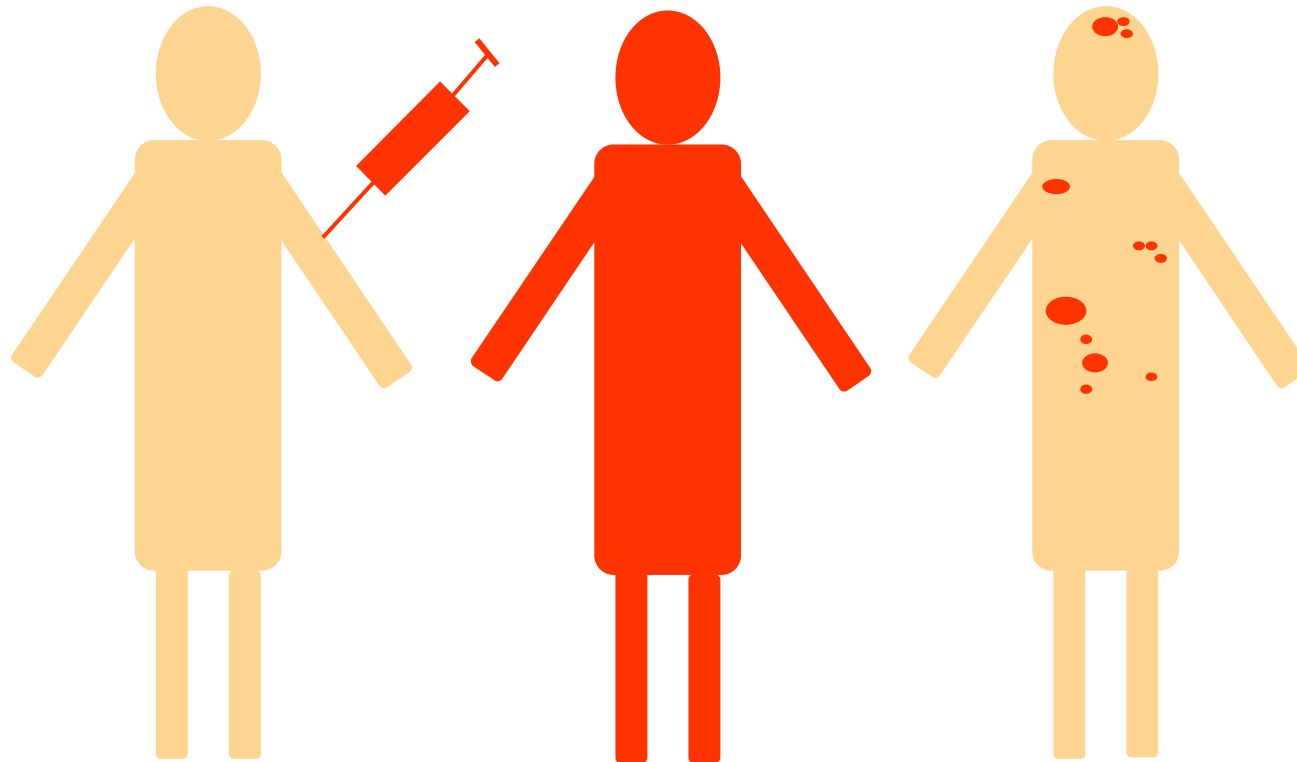
**Chelate/Linker**  
(e.g. derivatives of DTPA, DOTA)

+

Effective killing  
**α - Emitters**  
(e.g. Ac-225, Bi-213, U-230, Th-226)



## Alpha-Immunotherapy is a targeted therapy



**Injection**

**Circulation**  
**Whole body distribution**

**Localization**

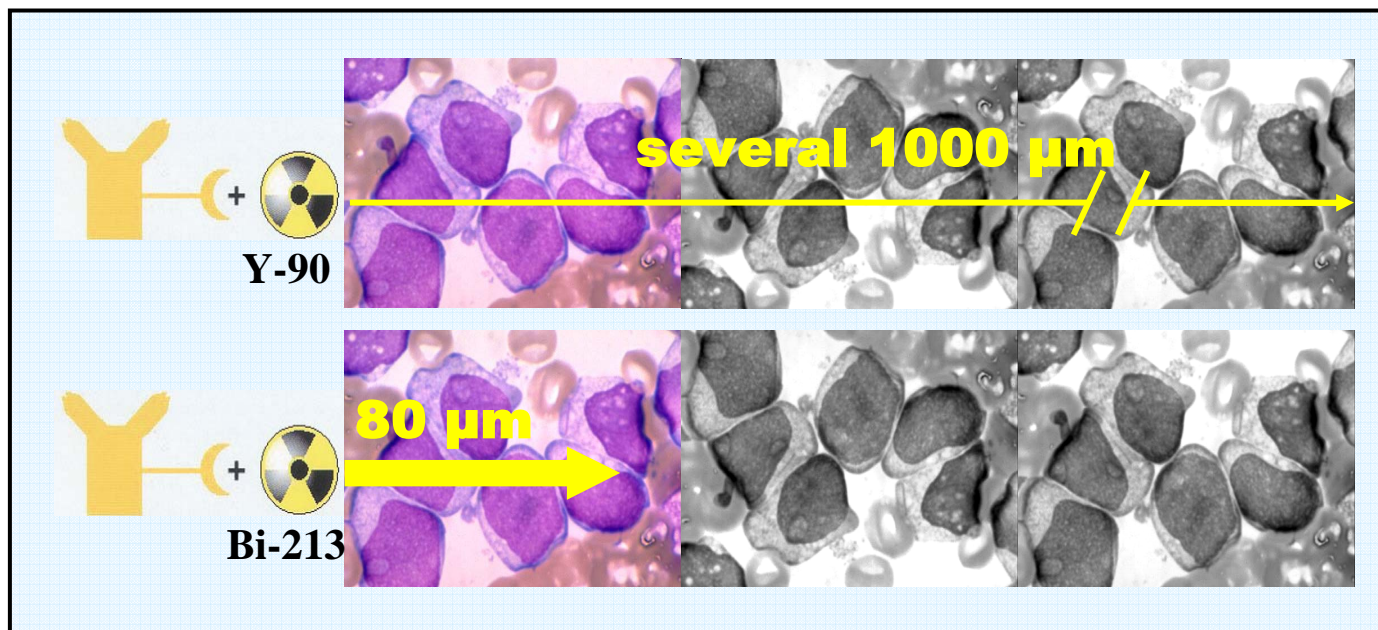


## Radioimmunotherapy $\beta$ and $\alpha$

### Beta particles:

Energy: 0.1 – 2.3 MeV, Range in soft tissue: 0.5 - 12 mm

Corresponding number of cells:  $\approx 10$ -1000



### Alpha particles:

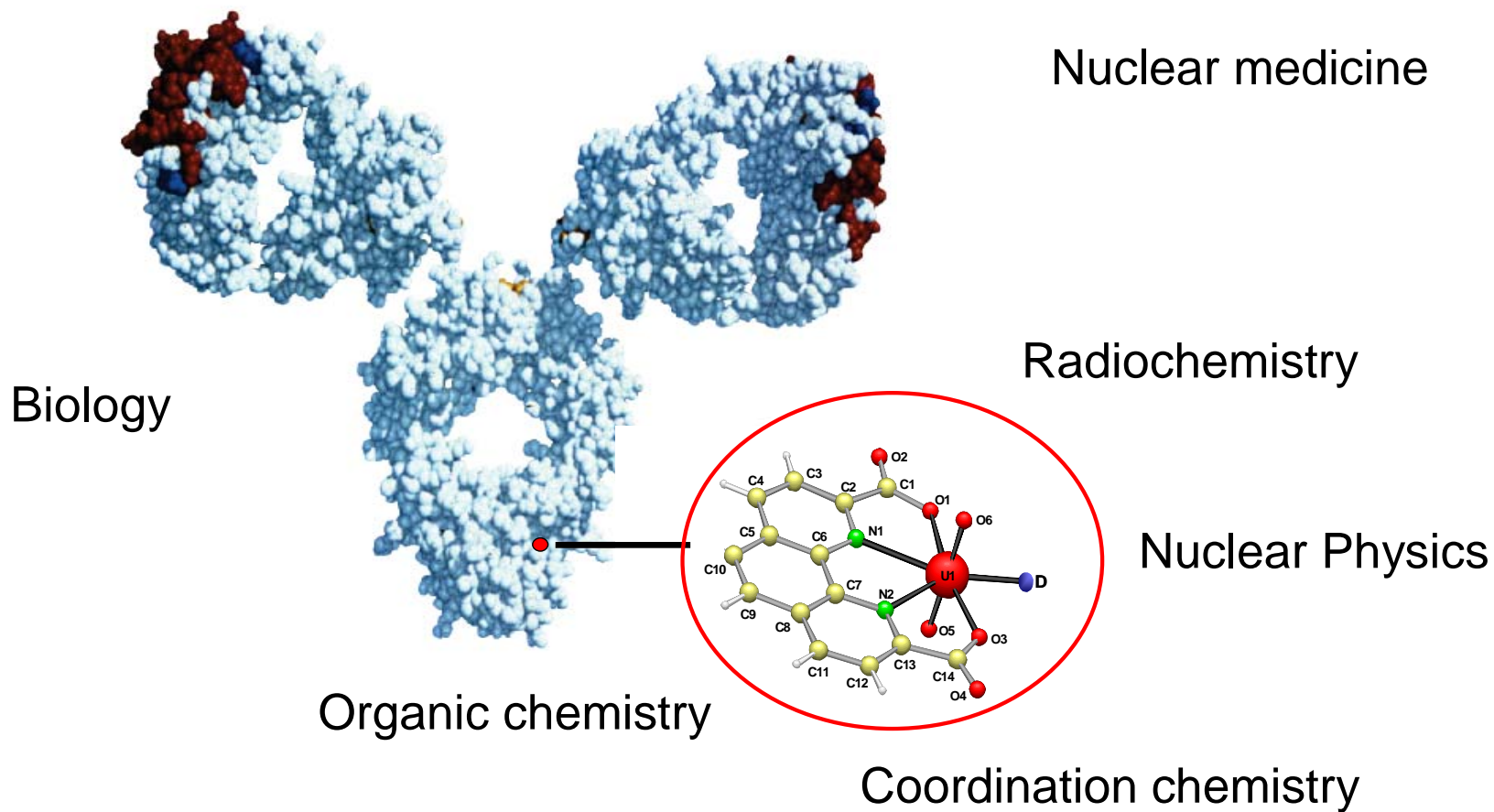
Energy: 4-9 MeV, Range in soft tissue: 50-90  $\mu\text{m}$

Corresponding number of cells:  $\approx 2$ -10

**=> Alpha particles deposit a high energy within a short range resulting in highly effective and selective cell kill**



## Alpha-immunotherapy – a multidisciplinary approach



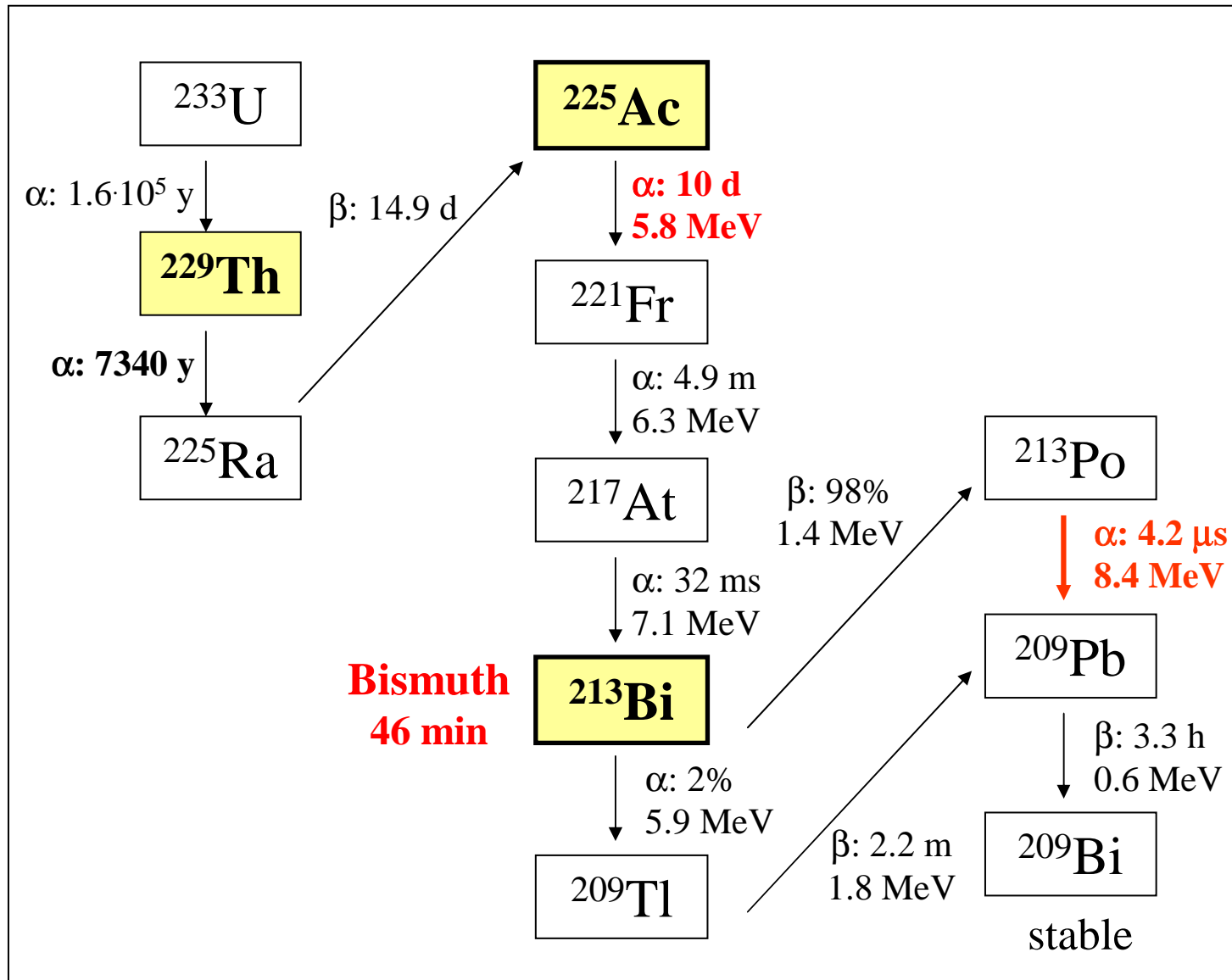


## *Alpha-immunotherapy at ITU:*

- Production of the alpha emitters Ac-225/Bi-213
  - *radiochemical process*
  - *cyclotron driven process*
- Pre-clinical studies *in vitro* and *in vivo*
  - *colony assays*
  - *spheroid studies*
  - *animal testing*
- Support of clinical trials
  - *leukemia*
  - *brain tumours*
  - *melanoma*



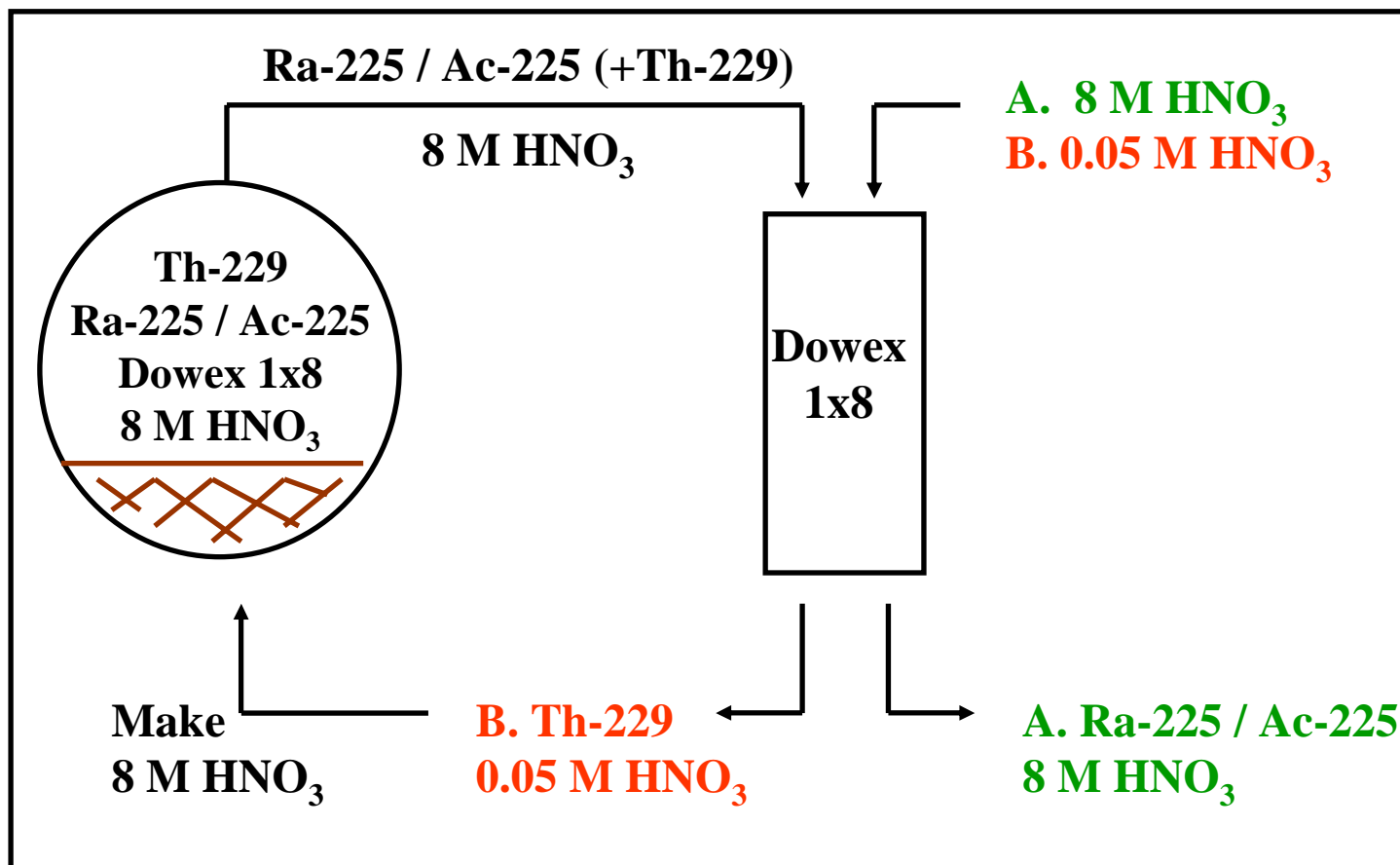
# The Ac-225/Bi-213 system







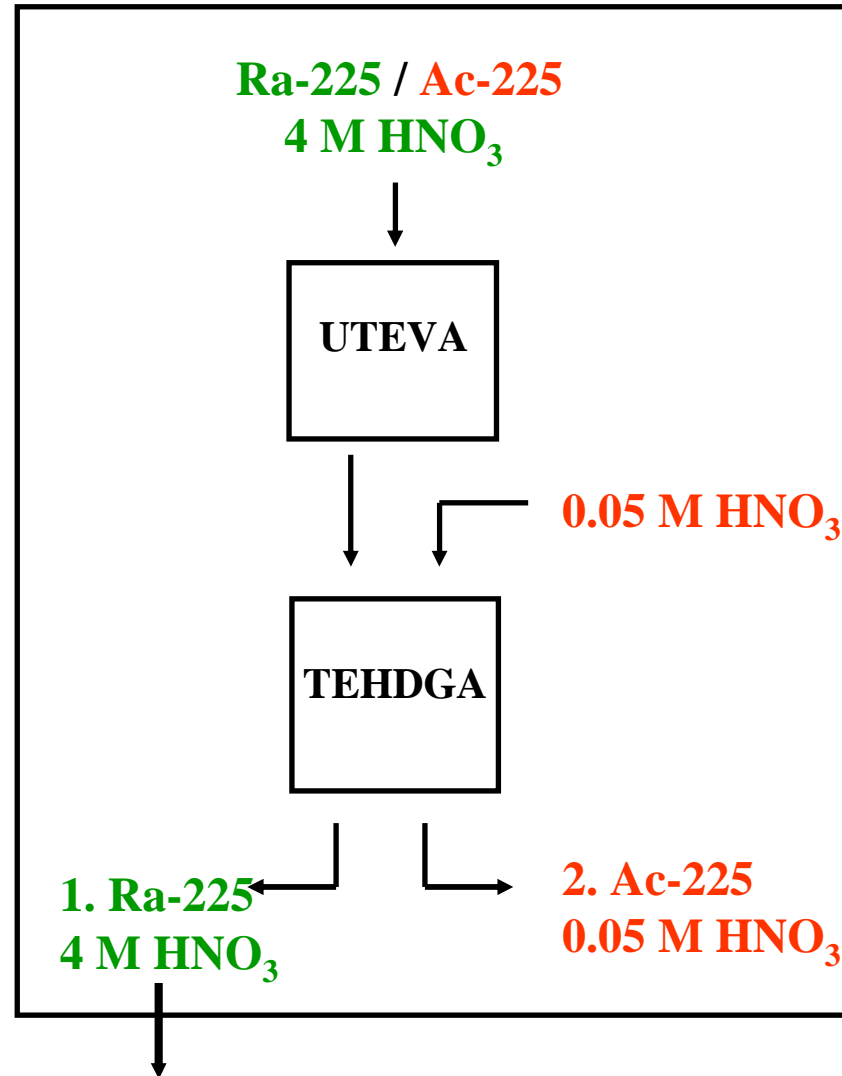
## Th-229 / Ra-225, Ac-225 separation Anion exchange







## Ra-225 / Ac-225 separation Extraction chromatography

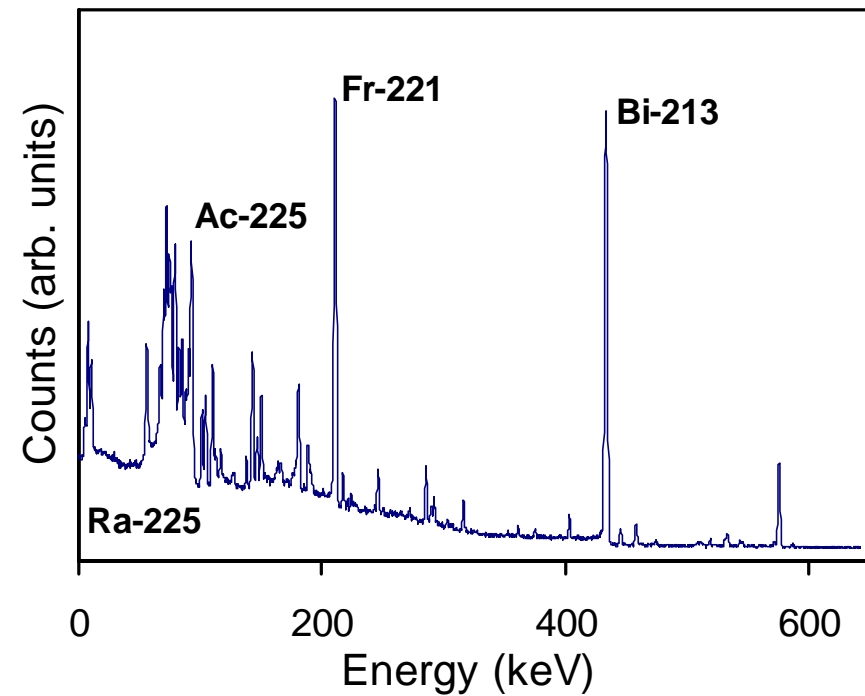
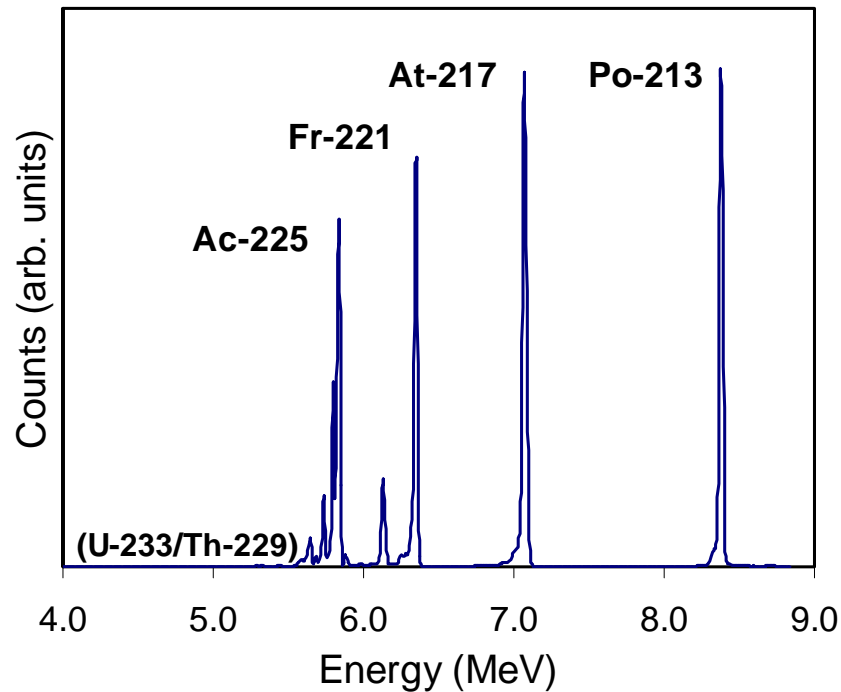


additional Ac-225 after approx. 3 weeks storage



## Quality control:

- Alpha-, gamma-spectrometry
- ICP-MS

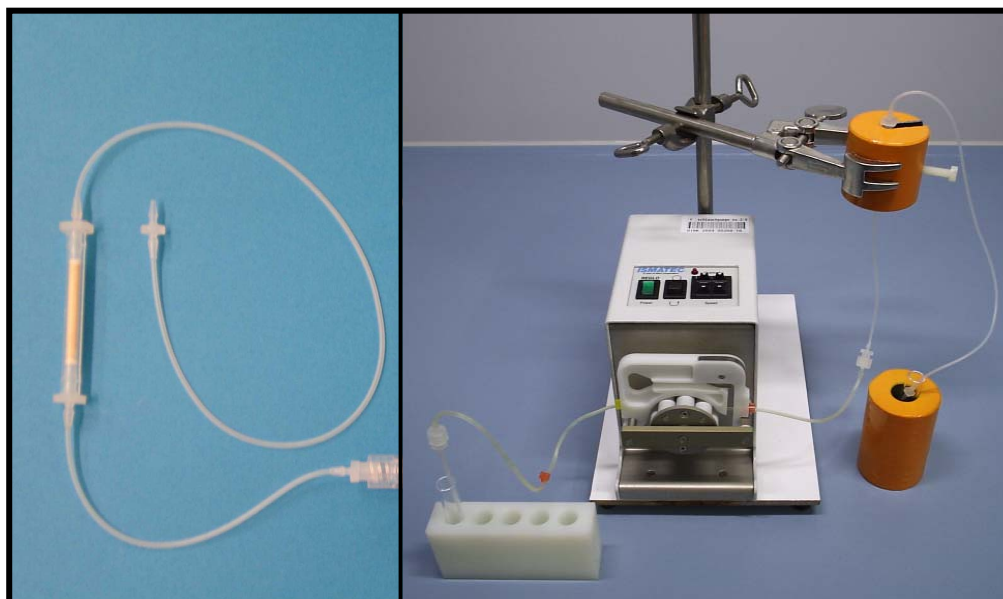


Overall yield of separation process: > 98%



## Annual production of Ac-225/Bi-213 at ITU

- Radiochemical separation from Th-229:  
=> max. 24 production runs per year with approx. 350 mCi Ac-225 in total



Shipments from ITU until today: > 270 generators = > 4 Ci Ac-225

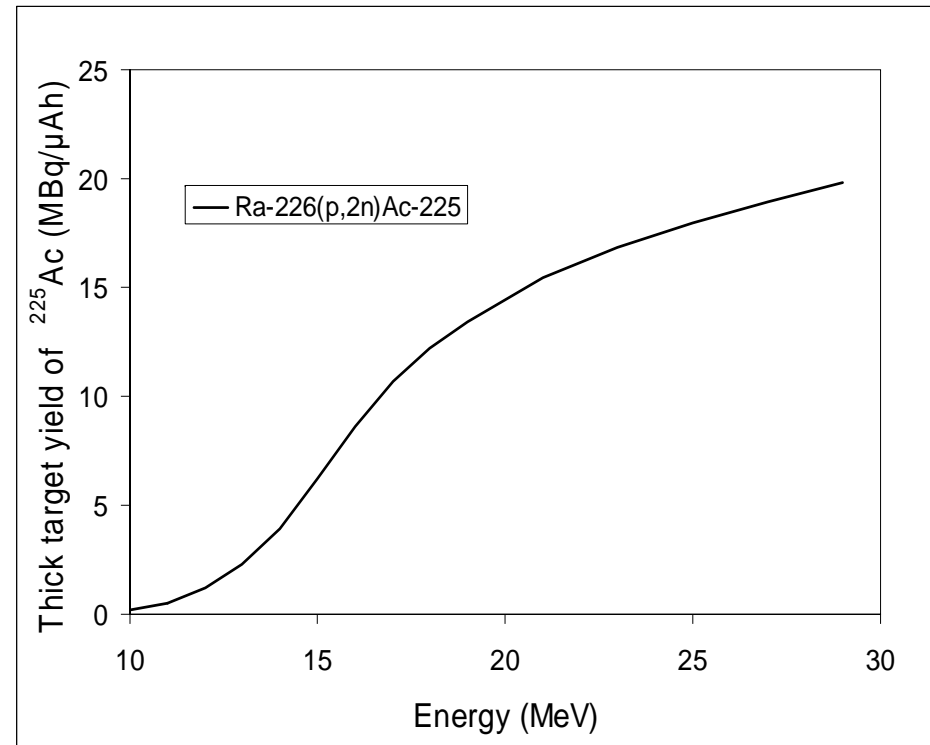
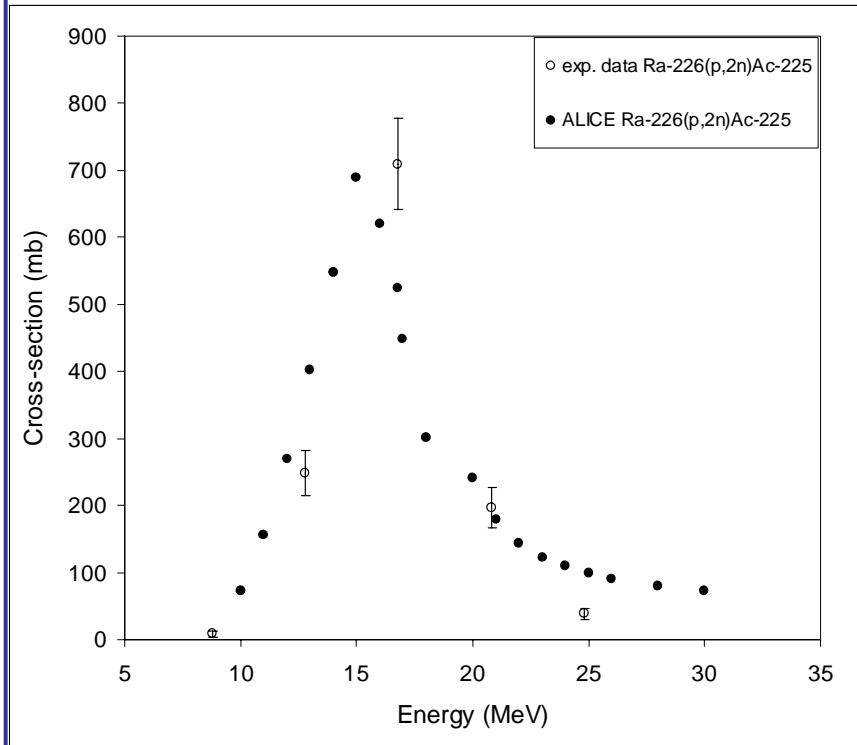
90% of Bi-213 patients (n = 131) treated world-wide to date  
have received Bi-213 produced at ITU

Currently only two other Ac-225/Bi-213 producers world-wide  
(ORNL, USA and Obninsk, Russia)



## Cyclotron production of Ac-225/Bi-213

Activation cross-sections and thick target yields for Ra-226(p,2n)Ac-225

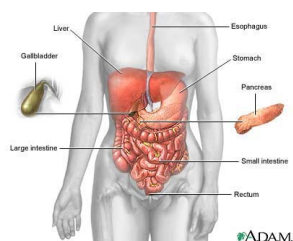


=> Cyclotron driven technology developed by ITU improves availability of Ac-225/Bi-213

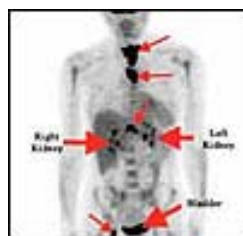
=> Widespread clinical application feasible



## Pre-clinical studies and clinical trials



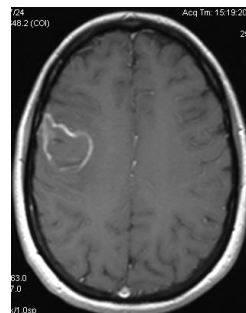
**Bladder carcinoma**  
(Munich)



**Breast Cancer**  
(Baltimore)



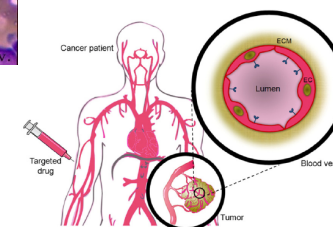
**Melanoma**  
(Sydney)



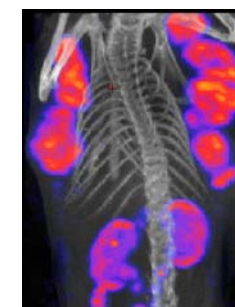
**Brain tumours**  
(Phase I, Basel)



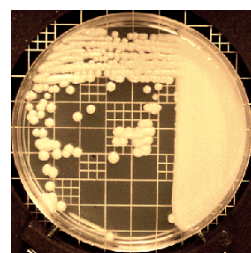
**leukemia**  
(MSKCC, New York)



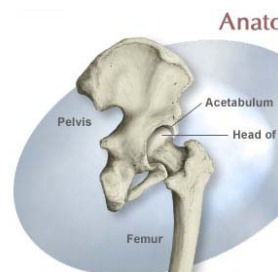
**anti-vascular therapy**  
(ETH, Neri)



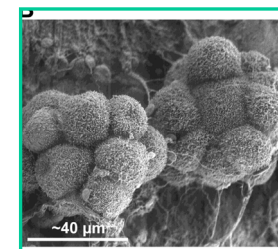
**Ovarian - folates**  
(PSI, Schibli)



**Infectious diseases**  
(New York)



**Multiple myeloma**  
(Nantes)



**Ovarian - mAbs**  
(Gothenburg)

**ITU**  
 **$\alpha$ -emitting**  
**isotopes**

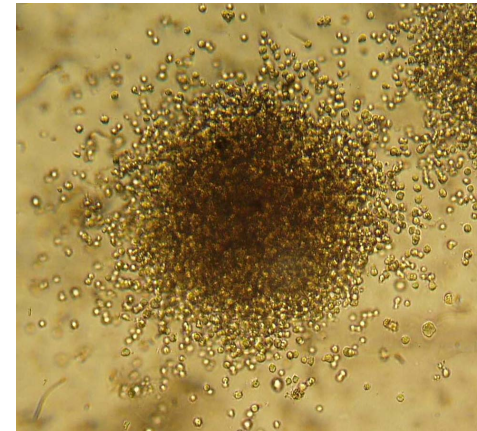
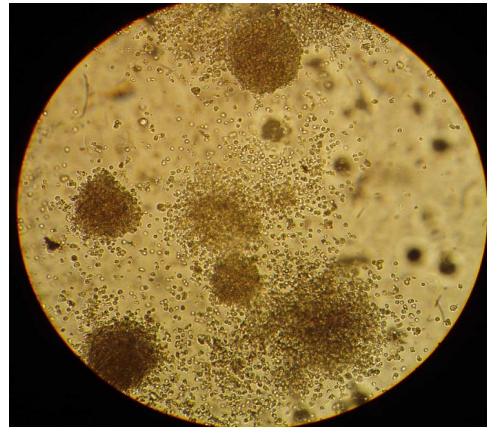




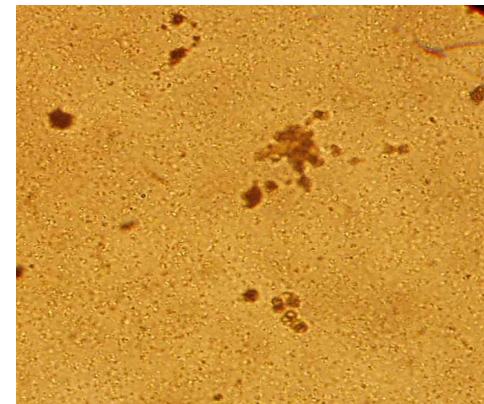
## *In vitro* - clonogenic assay

Determination of the capacity of cells to proliferate and form tumor cell colonies after radiation treatment

Control

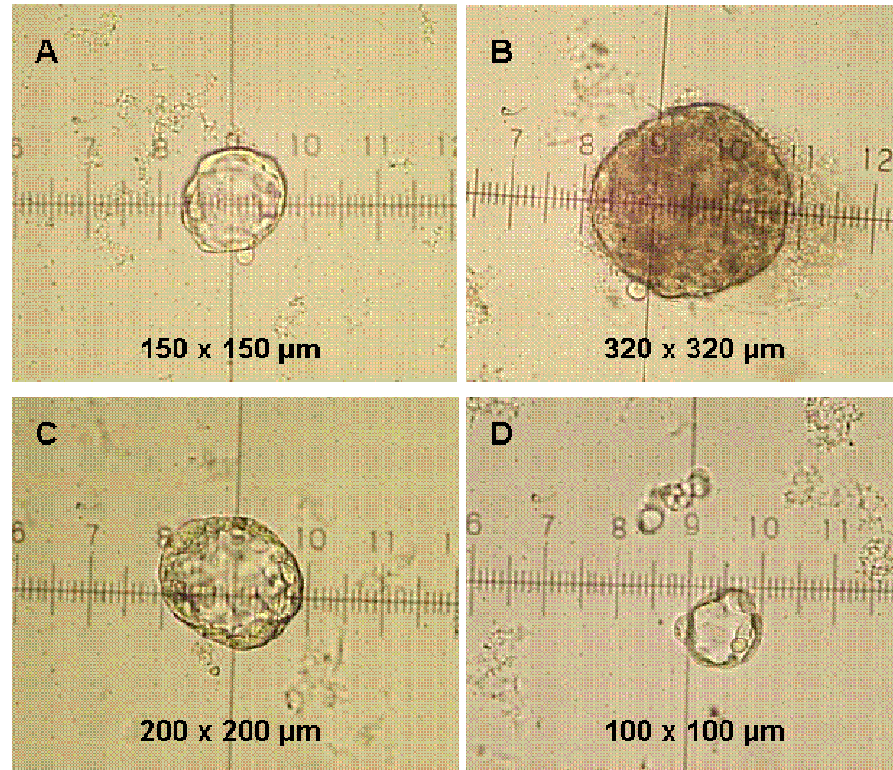


$^{213}\text{Bi}$ - anti-CD20 treatment





## *In vitro* - Breast cancer spheroids as model for solid tumors



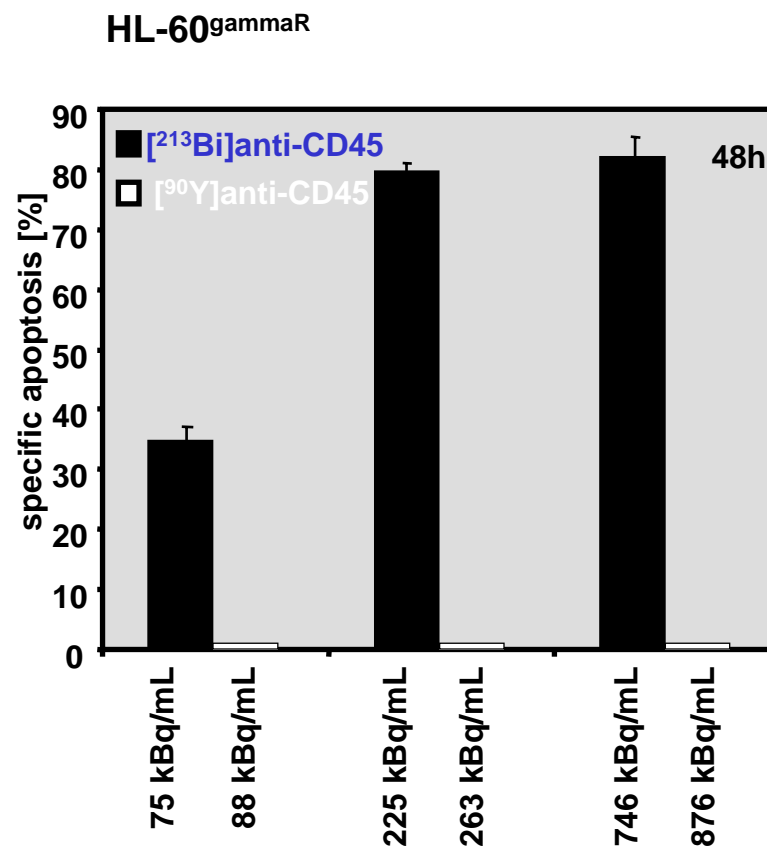
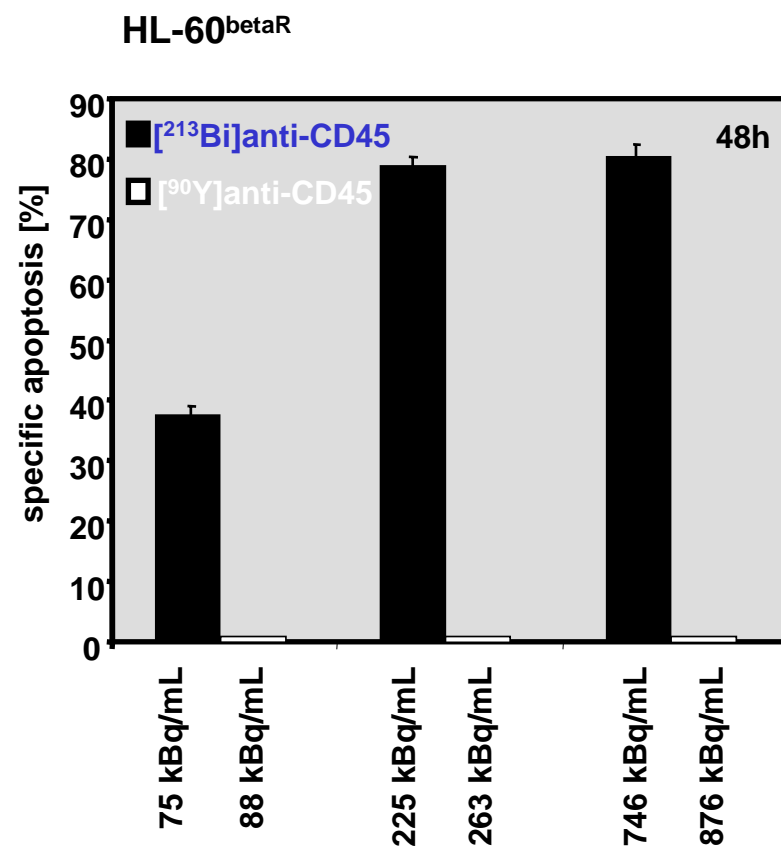
*Breast cancer spheroids with and without treatment (A: spheroid before treatment, B: untreated control spheroid after 3 weeks, C: 0.6 MBq/ml  $^{213}\text{Bi}$ -Herceptin, D: 1.2 MBq/ml  $^{213}\text{Bi}$ -Herceptin).*





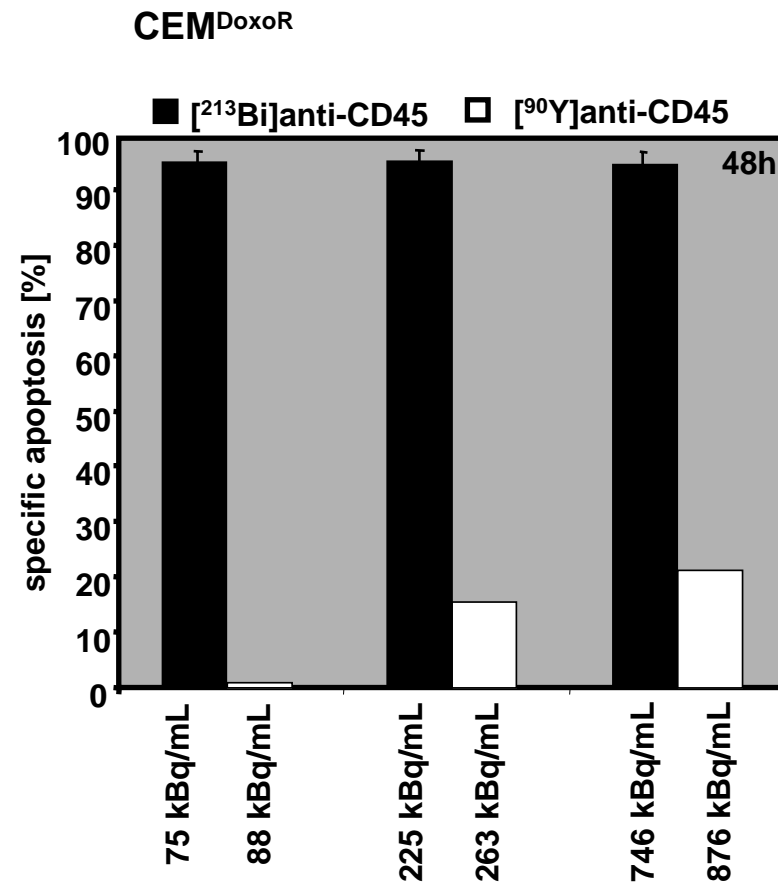
## *In vitro* – AIT breaks chemo- and radiation-resistance in leukemia cells

[<sup>213</sup>Bi]anti-CD45 and [<sup>90</sup>Y]anti-CD45 induces apoptosis in beta-radiation resistant HL-60<sup>betaR</sup> and gamma-radiation HL-60<sup>gammaR</sup> leukemia cells



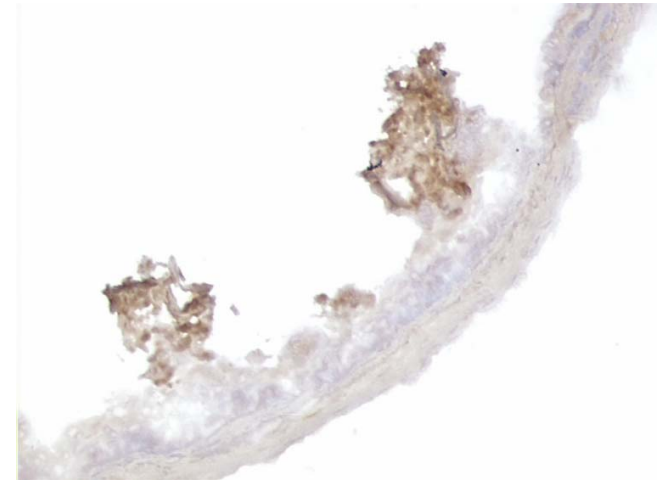


$[^{213}\text{Bi}]$ anti-CD45 induces apoptosis also  
in doxorubicin-resistant CEM<sup>DoxoR</sup> leukemia cells





*In vivo* – animal testing  
(ITU – Klinikum rechts der Isar, Munich)



Bladder carcinoma  
xenograft

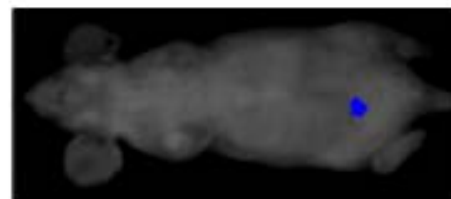
*Pre-clinical studies on bladder carcinoma*

- Conventional therapy: resection of main tumour, followed by local chemotherapy to eradicate residual tumour cells
- limitation: frequently resistance to chemotherapy leads to recurrence (up to 40% within 5 years)
- targeted alpha therapy can kill chemo-resistant cells

=> treatment strategy: replacement of local chemotherapy by local Bi-213 therapy post-resection



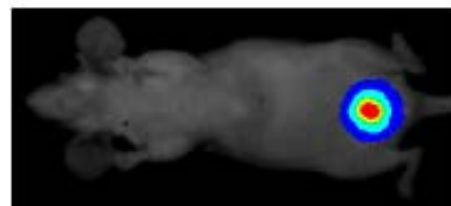
## In vivo imaging of tumour growth using bioluminescence



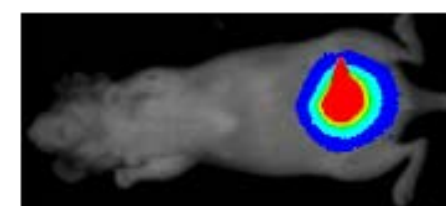
Tag 7 p. Zellinstillation



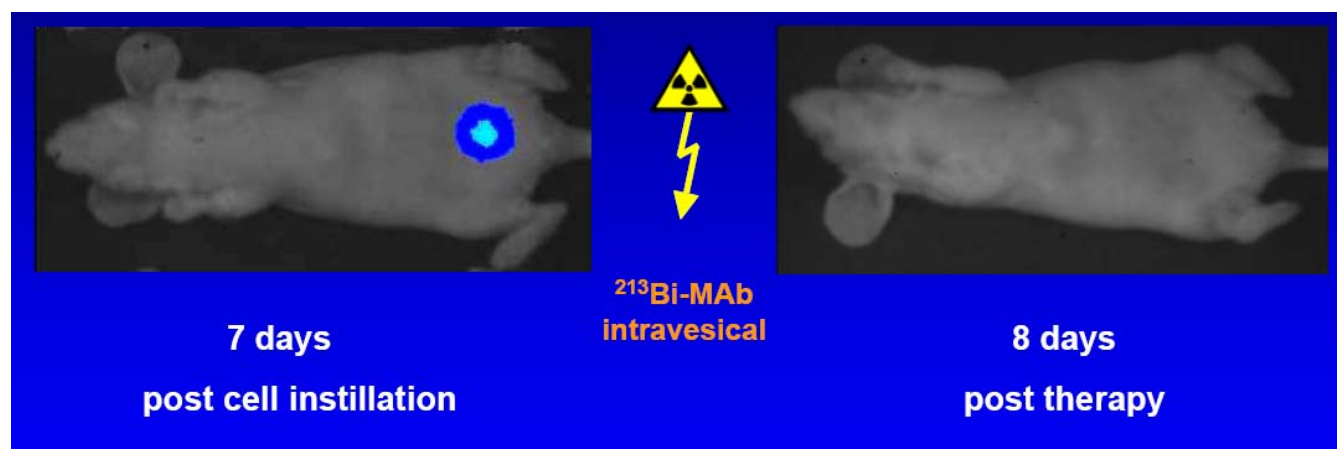
Tag 14 p. Zellinstillation



Tag 21 p. Zellinstillation

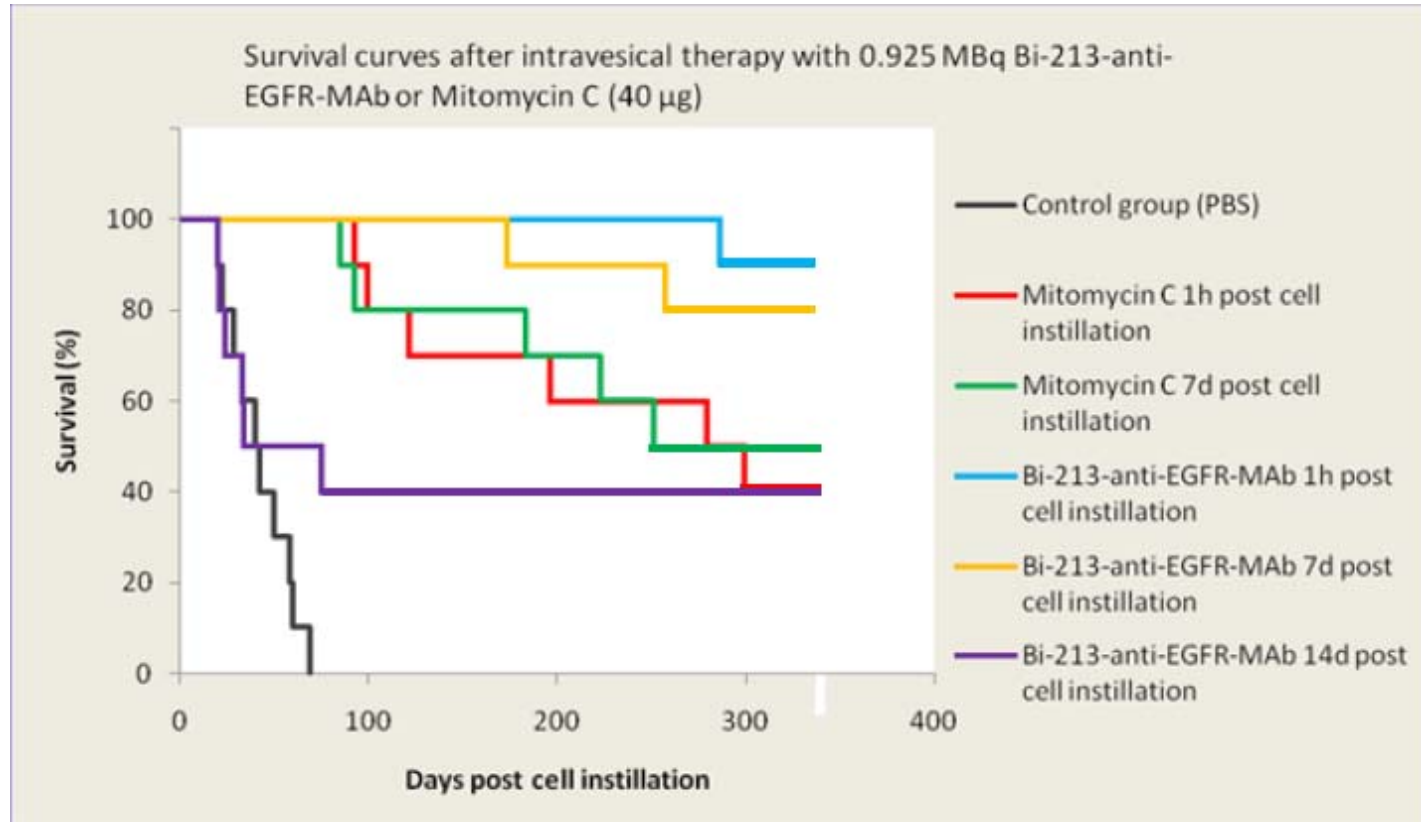


Tag 28 p. Zellinstillation





## Survival: AIT vs. chemotherapy





## Clinical trials: Alpha-Immunotherapy of leukemia ITU + Memorial Sloan Kettering Cancer Center, New York

### Phase I:

- 18 patients with relapsed and refractory AML (acute myelogenous leukemia) or CML (chronic myelomonocytic leukemia)
- 0.3 - 1.0 mCi/kg body weight of  $^{213}\text{Bi}$ -HuM195 (anti-CD33)
- no significant toxicity
- 14/18 patients responded

*J. Jurcic, Blood 2002*

### Phase I/II (ongoing):

- elimination of residual disease after partial cytoreduction using cytarabine
- >30 AML patients treated so far
- 0.5 – 1.25 mCi/kg body weight of  $^{213}\text{Bi}$ -HuM195
- MTD 1 mCi/kg
- 24% of patients receiving 1 mCi/kg (n=25) responded (2 CR, 2 CRp, 2PR)



**EUROPEAN COMMISSION**  
DIRECTORATE-GENERAL  
**Joint Research Centre**

## ITU - University Hospital Basel



Clinical study on brain tumors started in 2007

Local application of  $^{213}\text{Bi}$ -labeled peptide before surgery to improve demarcation of tumour from healthy brain tissue

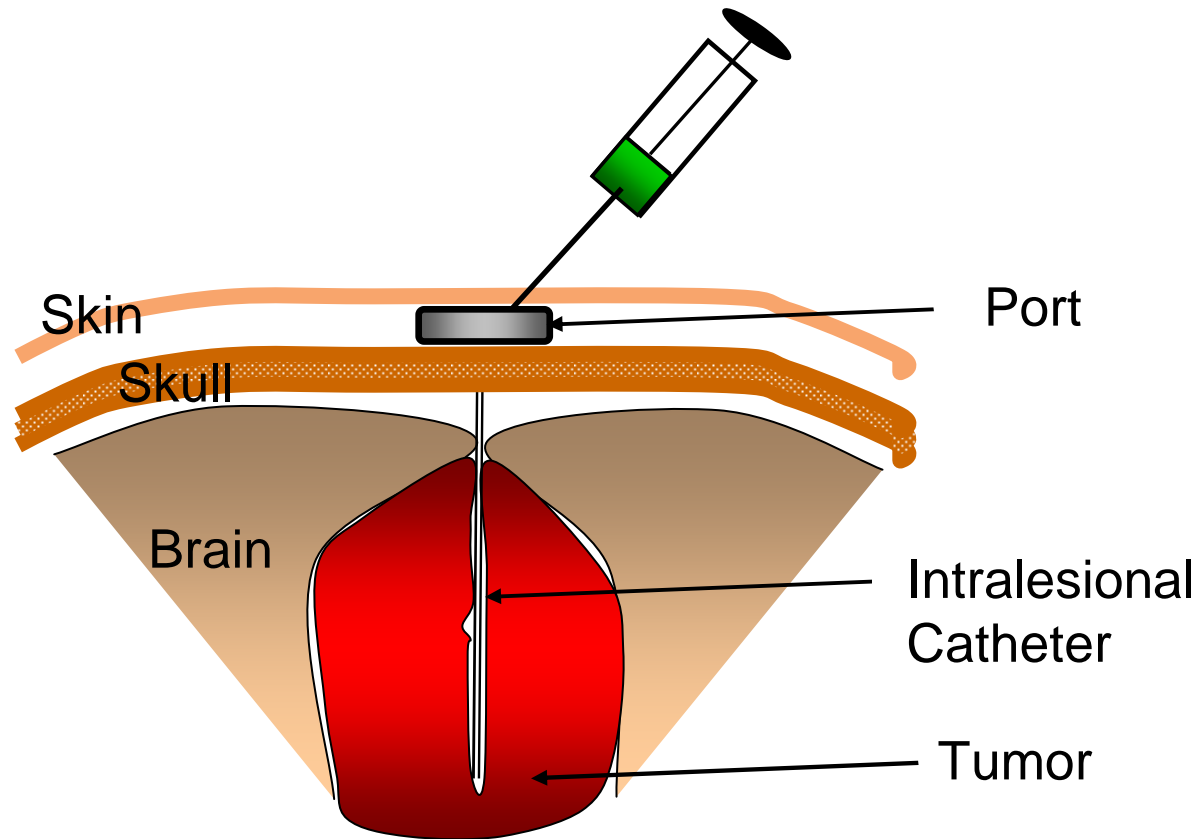
5 patients treated so far







## Application of $^{213}\text{Bi}$ -DOTA-substance P

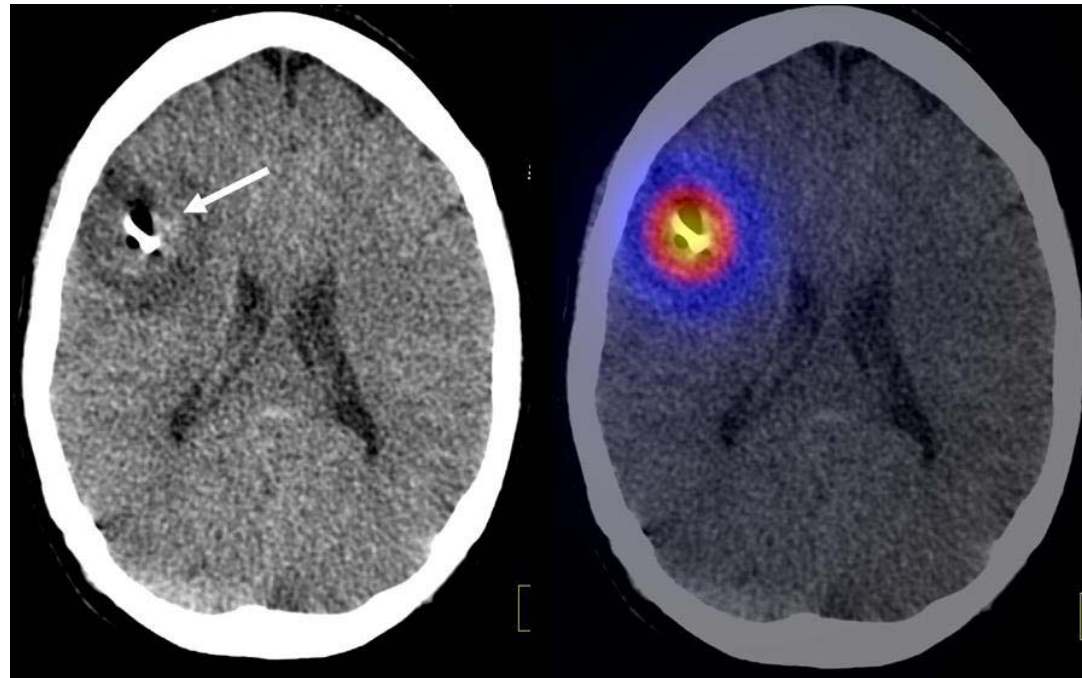




## Intermediate results

CT before injection

fused CT/SPECT after  
 $^{213}\text{Bi}$  injection



=> selective accumulation of alpha emitter

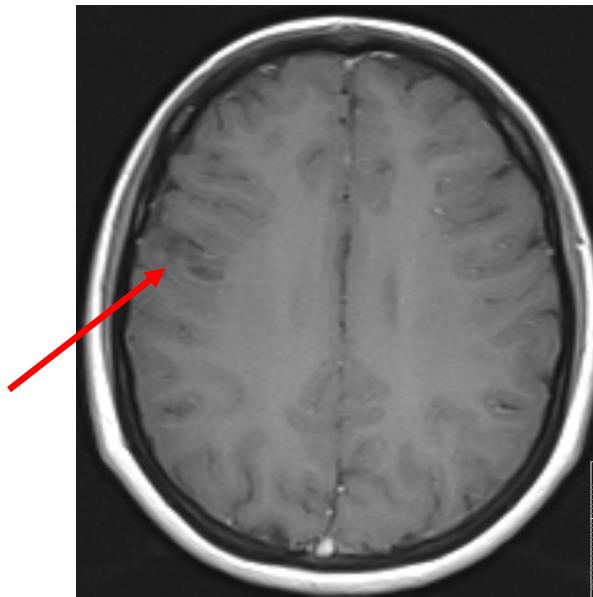


Patient IV:  
whole body  
scintigraphy  
30 min p.i.

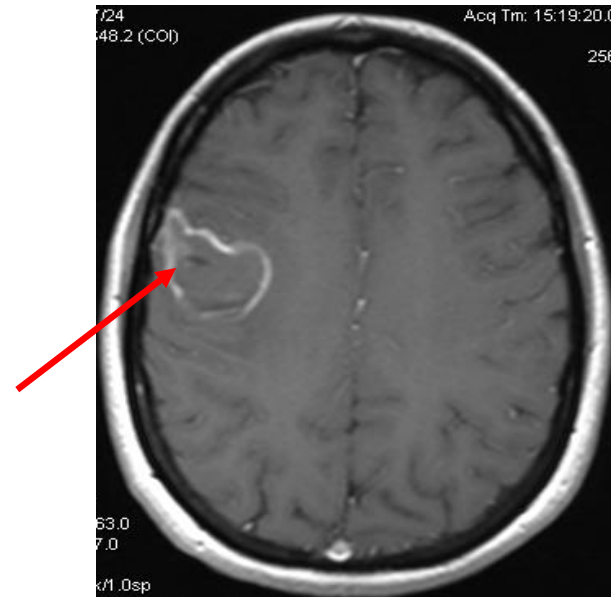


*Secondary endpoint: therapeutic efficacy (I)*

Case IV – WHO Grade II Glioma (34 y, female)



Pre-therapy: diffuse  
tumour boundaries  
(09/2007)

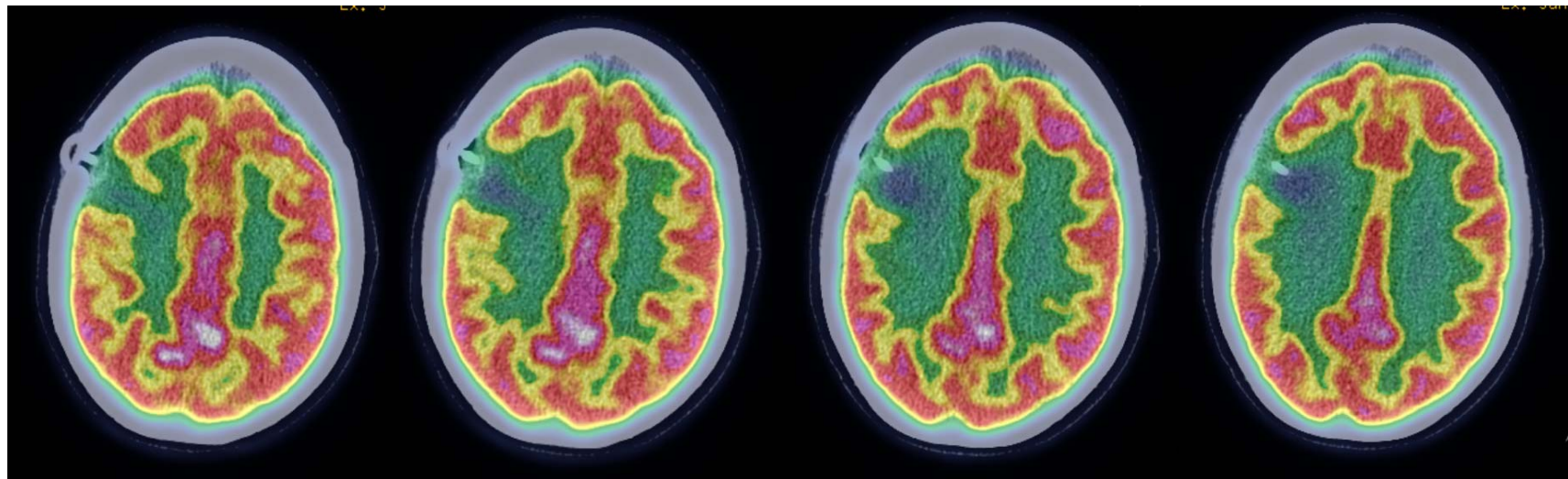


Post-  $^{213}\text{Bi}$ -therapy:  
clear demarcation of  
tumour (10/2007)



$^{18}\text{F}$ -FDG-PET directly prior to resection

16 weeks post therapy

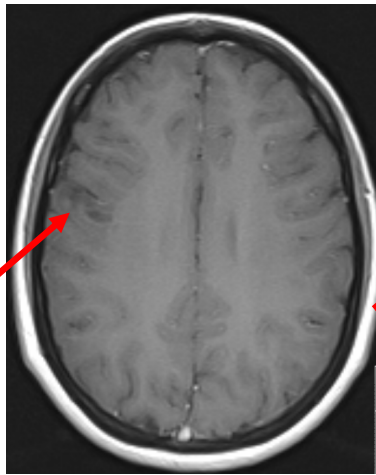


=> no significant uptake in tumour region

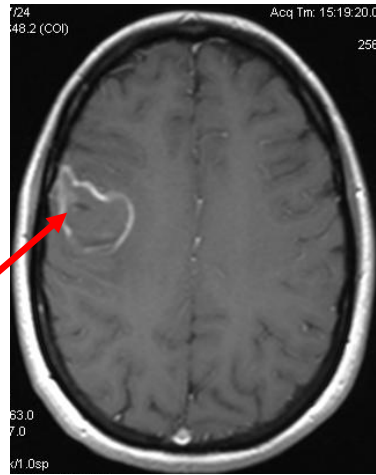


*Secondary endpoint: therapeutic efficacy (II)*

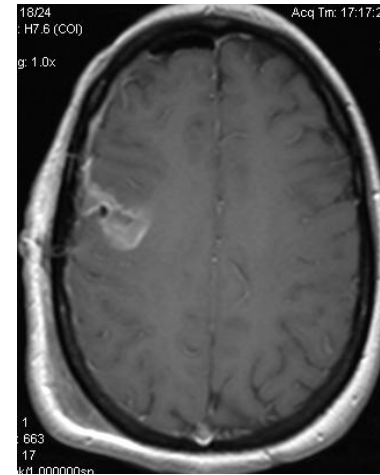
Case IV – WHO Grade II Glioma (34 y, female)



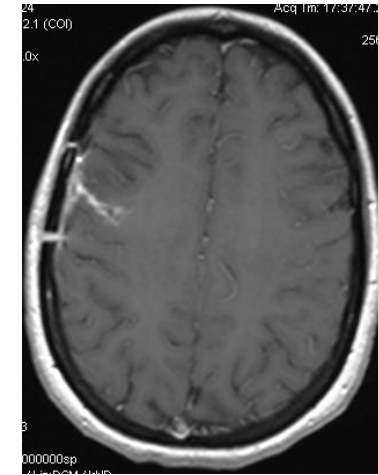
Pre-therapy: diffuse  
tumour boundaries  
(09/2007)



Post-  $^{213}\text{Bi}$ -therapy:  
clear demarcation of  
tumour (10/2007)



Early post surgery  
(01/2008)



2 months post surgery  
(03/2008)

**=> patient is tumour and symptom-free (10/2008)**

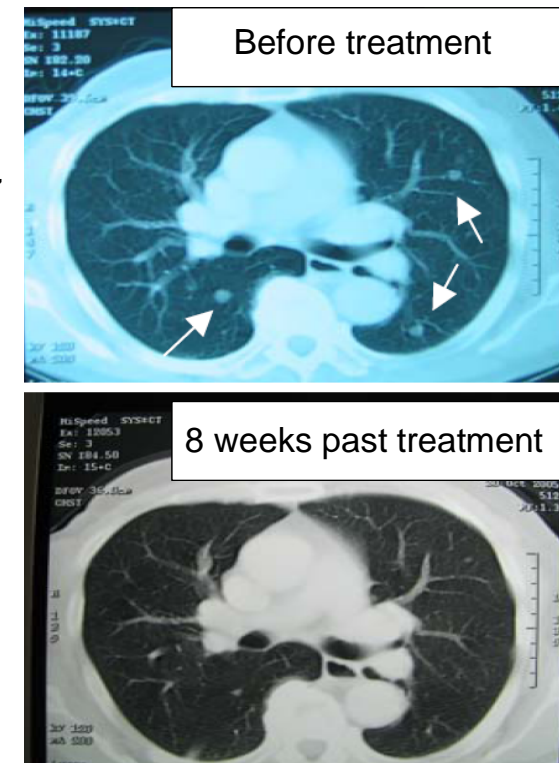




*Phase I study on grade IV malignant melanoma / in-transit metastases  
using Bi-213 labelled mAb 9.2.27*

Results:

- 48 patients treated with 1.5 to 27 mCi Bi-213-9.2.27
- 12% partial response, 50% stable disease, 38% progressive disease
- no signs of adverse effects



Regression of lung nodules in patient #18  
8 weeks after therapy with  $^{213}\text{Bi}$ -9.2.27





Regression of two subcutaneous melanoma metastases on the chest in patient #18 after  $^{213}\text{Bi}$ -9.2.27 therapy

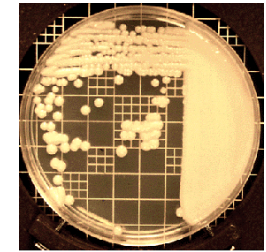


Patient #7 received a single injection of Bi-213-9.2.27  
=> 20 of 21 melanomas on the leg completely regressed,  
with no local recurrences at 12 months post-treatment  
(original tumour size is shown as a blue ring).



## Alpha-Immunotherapy of Fungal, Bacterial and Viral Infections

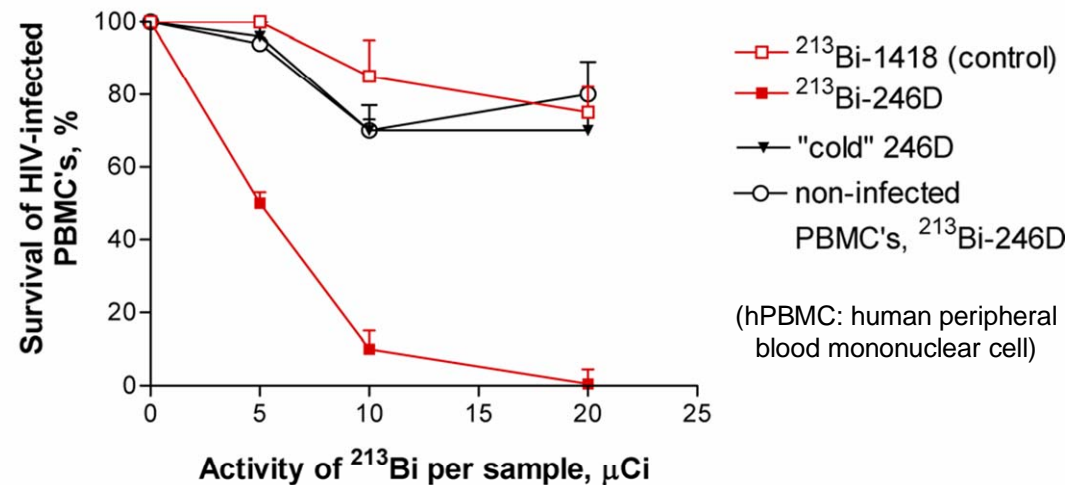
ITU - Albert Einstein College of Medicine, NY, USA



*Cryptococcus neoformans*  
Yeast extract glucose agar

### First report on targeting and elimination of HIV-infected cells using Bi-213

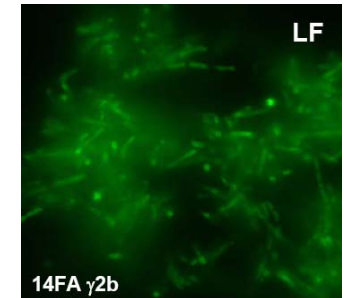
*In vitro*: Specific and highly effective killing of HIV infected cells using  $^{213}\text{Bi}$ -anti-gp41



*In vivo*: Treatment of infected mice with  $^{213}\text{Bi}$ -246D effectively reduced number of HIV1-infected cells by 300-fold, no acute toxicity observed in treated mice.

=> World-wide first clinical study on the treatment of HIV infections using Alpha-immunotherapy planned

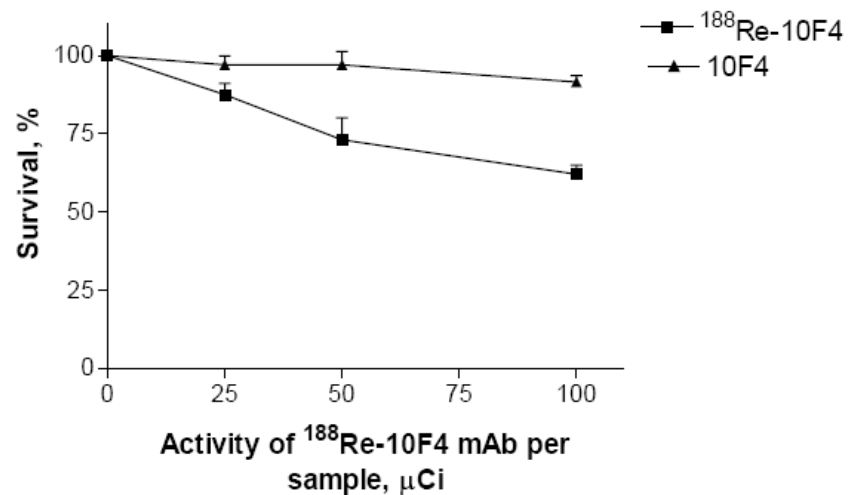
itu



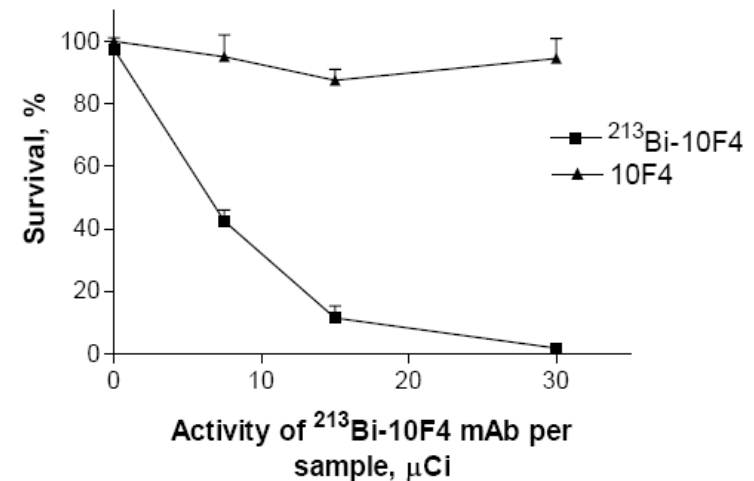
### *Bi-213 therapy for anthrax infections (I)*

- *Bacillus anthracis* is the causative agent of anthrax
- *Bacillus anthracis* produces three polypeptides which act in a binary fashion to make up the anthrax toxin: protective antigen (PA), lethal factor (LF) and edema factor (EF)
- mAb 10F4 is specific to PA, 14FA is specific to LF
- *in vitro* studies show that  $^{213}\text{Bi}$ -10F4 kills bacterial cells more effectively than  $^{188}\text{Re}$ -10F4 or cold mAb

A.



B.

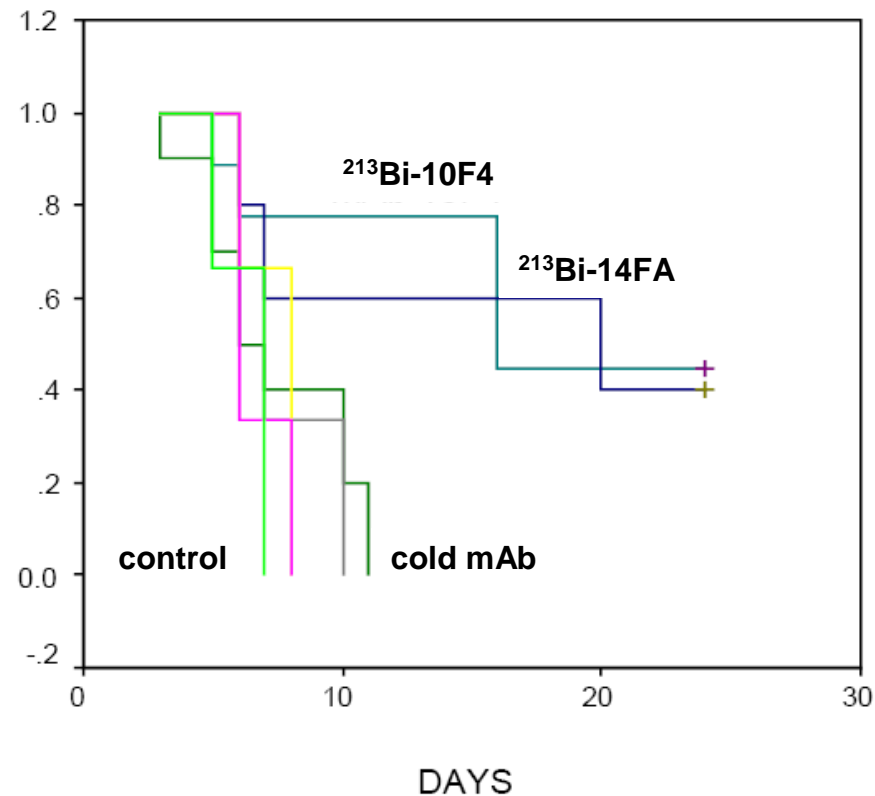




### *Bi-213 therapy for anthrax infections (II)*

- *in vivo* studies show that  $^{213}\text{Bi}$ -10F4 and -14FA prolonged survival of A/JCr mice infected with *B. anthracis*

Survival of mice infected with  $10^4$  *bacillus anthracis* cells 1 h prior to treatment



Johanna Rivera, Antonio S. Nakouzi, Alfred Morgenstern, Frank Bruchertseifer, Ekaterina Dadachova and Arturo Casadevall: Radiolabeled antibodies to *Bacillus anthracis* toxins are bactericidal and therapeutic in experimental anthrax, *submitted*



## *Some conclusions*

- Alpha-immunotherapy is a novel and promising approach for the treatment of cancer and infectious diseases
- Pre-clinical studies are ongoing in various fields
- The first clinical data are promising
- More studies required before routine application



## Alpha-Immunotherapy at ITU 2008

Christos Apostolidis

Frank Bruchertseifer  
Alfred Morgenstern  
Alexander Romanow  
Mirjam Weiss

Stefanie Kannengiesser  
Gilles Montavon  
Urska Repinc

*Thank you for your attention!*